BIOACCUMULATION OF TOTAL MERCURY AND MONOMETHYLMERCURY IN THE EARTHWORM *EISENIA FETIDA*

DENNIS T. BURTON[1,*], STEVEN D. TURLEY[1], DANIEL J. FISHER[1],
DONALD J. GREEN[2] and TOMMY R. SHEDD[3]

[1] *University of Maryland, Wye Research and Education Center, P.O. Box 169, Queenstown, MD 21658;* [2] *U.S. Army Garrison, Directorate of Safety, Health and Environment, Building E5771, Aberdeen Proving Ground, MD 21010;* [3] *U.S. Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702*

(*author for correspondence, e-mail: dburton@umd.edu; Tel.: +410-827-8056, Fax: +410-827-9039)

Abstract. Bioaccumulation factors (BAFs) for inorganic mercury in earthworms are usually <1; however, factors up to ~10 have been reported. Little information is available concerning the bioaccumulation of organic mercury in earthworms from actual contaminated soils and thus there has been uncertainty in the risk characterization phase of ecological risk assessments of mercury-contaminated sites. This study was initiated to determine the rate of uptake and bioaccumulation of total mercury (T-Hg) and monomethylmercury (MMHg) in *Eisenia fetida* from soils which have been contaminated with mercury for approximately 30 years. The study consisted of a 28-day uptake phase in three mercury-contaminated soils and one soil with background concentrations of mercury followed by a 14-day depuration phase in background soil only. Total mercury concentrations in the study soils ranged from 85 to 11,542 μg kg^{-1} dry weight soil; MMHg concentrations ranged from 1.12 to 7.35 μg kg^{-1} dry weight soil. Time to 90% steady states for T-Hg ranged from 36 to 42 days. A steady state did not occur for any of the MMHg exposures during the 42-day study; estimated time to 90% steady state varied from 97 to 192 days. BAFs for T-Hg ranged from 0.6 to 3.3. BAFs for MMHg ranged from 175 to 249. The BAFs for T-Hg and MMHg were larger in earthworms exposed to the lower contaminated soils and smaller in the higher mercury-contaminated soils. The absolute concentrations of T-Hg and MMHg bioaccumulated in *E. fetida*, however, were higher in the earthworms exposed to the higher mercury soils and lower in the less mercury-contaminated soils.

Keywords: BAF, bioaccumulation, bioaccumulation factor, earthworm, ecological risk assessment, *Eisenia fetida*, mercury, monomethylmercury, soil

1. Introduction

Mercury is a naturally occurring element that is ubiquitous in the environment. The element exists in three valance states (0, +1, and +2) as well as in various inorganic and organic complexes. Elemental mercury (Hg^0) is the most common form found in nature. Biogenic emissions to the atmosphere are the most important processes of mercury re-distribution to the environment; anthropogenic emissions (e.g., fossil fuel combustion) account for 10 to 30% of the mercury emitted annually (Stein

REPORT DOCUMENTATION PAGE

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2006		

4. TITLE AND SUBTITLE

Bioaccumulation of Total Mercury and Monomethylmercury in the Earthworm Eisenia Fetida

5a. CONTRACT NUMBER

DAMD17-92-C-2066

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dennis T. Burton, Steven D. Turley, Daniel J. Fisher, Donald J. Green, and Tommy R. Shedd

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Maryland, Wye Research and Education Center, P.O. Box 169 Queenstown, MD 21658; US Army Garrison, Directorate of Safety, Health and Environment, Bldg. E5771, Aberdeen Proving Ground, MD 21010; US Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research and Materiel Command
504 Scott Street
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Unlimited distribution

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Bioaccumulation factors (BAFs) for inorganic mercury in earthworms are usually <1; however, factors up to ~10 have been reported. Little information is available concerning the bioaccumulation of organic mercury in earthworms from actual contaminated soils and thus there has been uncertainty in the risk characterization phase of ecological risk assessments of mercury-contaminated sites. This study was initiated to determine the rate of uptake and bioaccumulation of total mercury (T-Hg) and monomethylmercury (MMHg) in Eisenia fetida from soils which have been contaminated with mercury for approximately 30 years. The study consisted of a 28-day uptake phase in three mercury-contaminated soils and one soil with background concentrations of mercury followed by a 14-day depuration phase in background soil only. Total mercury concentrations in the study soils ranged from 85 to 11,542 μg kg-1 dry weight soil; MMhg concentrations ranted from 1.12 to 7.35 μg kg-1 dry weight soil. Time to 90% steady states for T-Hg ranged from 36 to 42 days. A steady state did not occur from any of the MMHg exposures during the 42-day study; estimated time to 90% steady state varied from 97 to 192 days. BAFs for T-Hg ranged from 0.6 to 3.3

15. SUBJECT TERMS

BAF, bioaccumulation, bioaccumulation factor, earthworm, ecological risk assessment, Eisenia fetida, mercury, monomethylmercury, soil

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	18	Carol O'Brien
U	U	U			19b. TELEPHONE NUMBER *(Include area code)* 301-619-7685

et al., 1996). The predominant form of mercury in the atmosphere is Hg^0 vapor (95 to 100%)(Munthre, 1994). The ultimate fate of atmospheric mercury is wet and dry deposition, of which the former is probably the most important (Seigneur *et al.*, 1999). Wet deposition can only occur after volatile Hg^0 has been oxidized to water soluble forms, such as divalent mercury (Hg^{2+}) (Munthre, 1994). When deposited to surface soil, mercury is retained primarily as complexes of Hg^{2+} bound with sulfides, clay particles, and organic matter (Keating *et al.*, 1997; Loux, 1998).

Divalent mercury in soil can be methylated by anaerobic, and to a lesser extent, aerobic microorganisms to form primarily monomethylmercury (CH_3Hg^+). Dimethylmercury [$(CH_3)_2Hg$] formation, which is more common in marine sediments, can also occur in soil, but at lower concentrations (Stein *et al.*, 1996; Loux, 1998). Monomethylmercury formation is favored under acidic conditions in soils; $(CH_3)_2Hg$ formation is favored under neutral or alkaline conditions in the presence of a strong complexing agent (Stein *et al.*, 1996). The amount of methylmercury in soils is low relative to total mercury. According to Boudou and Ribeyre (1997), the normal percentage of total mercury in the form of methylmercury in soils ranges between 0.5 and 1.5%.

Elemental and inorganic mercury in general are less toxic to terrestrial organisms than methylmercury (Stein *et al.*, 1996). Likewise, metallic and inorganic mercury do not bioaccumulate in terrestrial organisms to the degree that methylmercury does (Boudou and Ribeyre, 1997). Bioaccumulation factors (BAFs) for total mercury in earthworms, which are important in the diets of vermivorous wildlife, are usually one or less; however, uptake factors up to ~10 have been reported (Cocking *et al.*, 1991; Fischer and Koszorus, 1992; Cocking *et al.*, 1994; Sample *et al.*, 1999). Limited studies of food chain transfer of mercury from contaminated surface soil to small mammals that consume earthworms as part of their diet indicate that inorganic mercury concentrations in biota do not exceed concentrations in the soil (Bull *et al.*, 1977; Talmage and Walton, 1993). In contrast to inorganic mercury, a number of studies have shown that methylmercury can bioaccumulate in birds and mammals, particularly in piscivorous wildlife (Wolfe *et al.*, 1998). With the exception of a study by Beyer *et al.* (1985) who demonstrated that methylmercury can bioaccumulate in earthworms, little information is available concerning the bioaccumulation of methylmercury in earthworms exposed to mercury-contaminated soils. This study was initiated to determine the uptake, depuration, and bioaccumulation of total mercury and monomethylmercury for an earthworm exposed to historically mercury-contaminated soil because of their importance in many temperate terrestrial ecosystems as a food source for small birds and mammals. The use of field soils contaminated with mercury over an extended period of time (i.e., naturally aged soil) should give a better estimate of mercury bioaccumulation for use in risk assessment than reference soils freshly spiked with mercury (Lock and Jannsen, 2003; Lanno *et al.*, 2004).

2. Materials and Methods

2.1. STUDY SOILS

Three contaminated soil samples, which contained a high, intermediate, and low concentration of mercury, were evaluated in the study. A soil with background concentrations of mercury was taken approximately 500 m from the contaminated site. The contaminated and background soil samples were taken from a former chemical warfare materiel decontamination area at Graces Quarters, Aberdeen Proving Ground, Maryland that had not been used since 1971. The three contaminated and background mercury soils, which were taken from the 0–15 cm horizon, were all Mattapeake/Mattapex soils.

An aliquot of each soil sample was taken for bulk chemical analyses, which included metals, volatile organics, base neutrals, acid compounds, pesticides/PCBs, herbicides, and explosives. The four soils were also characterized for cation exchange capacity, particle size distribution, nitrogen, and total organic carbon. All samples were placed in individual 4 mil plastic bags and kept cold (ice) in the field and during transport back to laboratory. All samples (less the aliquots used for chemical analysis) were stored in their original containers in the dark at 4 °C in the laboratory. The exposure phase of the study was initiated one week after the soil samples were collected.

The soil samples were prepared for testing via the procedures outlined in the ASTM (1998) standard guide for conducting laboratory soil bioaccumulation tests with lumbricid earthworms. All indigenous earthworms, cocoons, insects, and other debris were removed from the soils before the soils were sieved through a 6.35 mm stainless steel sieve and homogenized. Before the earthworms were placed in the soils, water content was adjusted to ~47% moisture content and pH was adjusted to ~5.8.

2.2. TEST ORGANISM

The lumbricid earthworm *Eisenia fetida* was used for all bioaccumulation tests. The earthworms were cultured in-house by the procedures given in ASTM (1998). Briefly, *E. fetida* was reared in a bedding of sphagnum peat moss with the pH adjusted to ~6 using calcium carbonate hydrated with reverse osmosis water. Moisture content was monitored on a weekly basis. Covered plastic trays were maintained so that there was no standing water in the bottom of the tray and the surface of the bedding was not dry. The trays were held under continuous lighting (~430 lumen m^{-2}) at 22 °C (±1 °C). The animals were fed fermented alfalfa pellets once or twice per week, depending on the number of individuals in a tray. The culture carrying capacity recommended in ASTM (1998) was followed. The bedding was periodically changed to prevent overcrowding. The bioaccumulation tests were initiated with sexually mature, fully clitellate adults.

2.3. EXPERIMENTAL PROCEDURES

The experimental design consisted of a 28-day uptake phase in the three contaminated soils and background mercury soil followed by a 14-day depuration phase. All earthworms exposed to the three contaminated and background mercury soils during the uptake phase were placed in background soil only during the depuration phase of the study. Earthworms from the background bioaccumulation experiment were placed in fresh background soil during the depuration phase. Three days prior to the earthworms being placed in the exposure soils, all earthworms were placed in homogenized background mercury soil (moisture content and pH adjusted as described above) to 'acclimate' to the soil matrix.

Twenty-four hours prior to the start of the exposure, all study earthworms including the background mercury earthworms were removed from the background soil and randomly placed in groups of 10 in polystyrene Petri dishes lined with moist filter paper to purge their gut contents. After purging for 24 h, each group of 10 earthworms was rinsed with reverse osmosis water, blotted gently by placing between layers of lint-free paper towels, and weighed. Each group of 10 earthworms was randomly loaded into 473 mL glass enclosed containers loaded with 400 g of each type of soil. The containers were fitted with Teflon®-lined lids pierced with a hole for ventilation. All test containers were randomly placed in an environmental chamber maintained at 22 °C ($\pm$ 0.2 °C) with continuous lighting of approximately 430 lumen m^{-2} at the surface of the soil. All 'acclimation' trays and purging dishes were also held in the same environmental chamber under the same test conditions.

At day 0 of the study, four replicates of soil from each of the three contaminated soils and background soil were analyzed for T-Hg and MMHg. Four replicates of 10 earthworms replicate^{-1} were randomly selected at day 0 for T-Hg and MMHg analyses in the background mercury soil only. In addition, four test vessels per soil type containing only soil were placed in the environmental chamber for T-Hg and MMHg analyses at day 28 of the uptake phase.

During the 28-day uptake phase, four randomly selected replicates of 10 earthworms replicate^{-1} were analyzed for T-Hg and MMHg in each contaminated soil and background mercury soil at days 1, 2, 4, 7, 14, 21, and 28. Four replicates of 10 earthworms replicate^{-1} were analyzed for T-Hg and MMHg from each contaminated soil and background soil at days 35 and 42 during the 14-day depuration phase conducted in the background soil. At each sample period, the earthworms in each replicate were counted (to determine survival), their guts purged for 24 h, and weighed as described below. At the end of the 28-day uptake phase, the earthworms in each remaining replicate were placed in fresh background mercury soil (soil replicates prepared from the original background mercury soil) and sampled at days 35 and 42 during depuration. Upon transfer to the background soil, all earthworms burrowed into the soil with no observable differences between earthworms in the treatments.

Soil temperature and percent moisture were monitored two times per week in a composite of four replicates from each of the four test soils during the 42-day test. Soil moisture was maintained at approximately 47% of field capacity (van Gestel *et al.*, 1992). Soil moisture was adjusted if necessary in all remaining replicates by reverse osmosis water. The earthworms were fed weekly during the uptake and depuration phases of the study (Gibbs *et al.*, 1996). A bolus of fermented alfalfa was added to a hole in the soil of the test vessel of each replicate at a rate of 350 mg g^{-1} earthworm week^{-1} as recommended by van Gestel *et al.* (1992). Excess food was removed after two days to prevent fungal growth. Soil pH was measured at the beginning of both the uptake and depuration phase in a composite of four replicates from each soil type.

2.4. MERCURY ANALYSES

The earthworms in each replicate were combined for chemical analyses. Each replicate was analyzed individually. Total mercury (T-Hg) and monomethylmercury (MMHg) analyses were made on the whole animal. After the earthworms in each replicate were purged for 24 h and weighed, they were placed in acid rinsed 40 mL glass vials with Teflon®-lined lids. The vials were refrigerated at 4 °C prior to being packed with blue ice and shipped overnight in polyfoam-lined containers to Brooks Rand, Ltd. (Seattle, Washington) for analysis. All soil samples ($\sim$10 g replicate^{-1}) were also placed in acid rinsed 40 mL glass vials with Teflon®-lined lids and treated in the same manner as the earthworms.

Total mercury in both the earthworm and soil samples was determined by cold vapor atomic fluorescence spectrophotometry. Briefly, the solid samples (both earthworm and soil) were digested with a 70:30 nitric:sulfuric acid solution and further oxidized with bromine chloride. The oxidation:digestion procedure converted all mercury species to Hg^{2+}. The samples were then reduced by tin chloride to form volatile Hg^0 (elemental mercury). The samples were purged with Hg-free nitrogen and the mercury collected and concentrated on a gold trap. The gold trap was then heated, thermally desorbing the mercury, which was swept by an inert carrier gas through an atomic fluorescence mercury detector. Peak area (fluorescence response) was measured (as elemental mercury) using a standard calibration curve.

Monomethylmercury was also determined by cold vapor atomic fluorescence spectrophotometry. The earthworms were digested in a potassium hydroxide/methanol solution. The soil samples were distilled in Teflon® distillation equipment. All samples were then ethylated forming a methyl-ethyl mercury derivative. The derivative was then purged onto a precollection trap. The trap was moderately heated under the flow of an inert carrier gas, releasing the mercury species. The mercury species were then separated using gas chromatography, after which they were pyrolitically broken down to Hg^0 prior to passing through an atomic fluorescence mercury detector. Peak area (fluorescence response) was measured (as elemental mercury) using a standard calibration curve.

The method detection limit (MDL) and practical quantitation limit (PQL) using the above analyses for T-Hg in both tissue and soil for a 5 g sample (wet weight) were both 0.1 ng g^{-1} dry weight. The MDL and PQL for MMHg in tissue were 1 and 5 ng g^{-1}, respectively. The MDL and PQL for MMHg in soil were 0.002 and 0.01 ng g^{-1}, respectively. Tissue and soil dry weight were determined gravimetrically.

2.5. DATA ANALYSES

A two-compartment (soil and earthworm) first order kinetic model was used to describe the movement of mercury in and out of the earthworm. For this model, changes in the concentration of mercury in the earthworm are described by the differential equation:

$$dC_w/dt = k_1 C_s - k_2 C_w \tag{1}$$

where: C_w = concentration of mercury in the earthworm; C_s = concentration of mercury in the soil; k_1 = uptake rate constant (day^{-1}); k_2 = depuration rate constant (day^{-1}); and t = time (day). With initial conditions of $t = 0$, $C_w = 0$, and C_s = constant, this equation has the simple solution of (Newman and Unger, 2003):

$$C_w = C_s(k_1/k_2)(1 - e^{-k_2 t}) \tag{2}$$

As the exposure time approaches infinity, the equation for the steady-state condition becomes:

$$C_w/C_s = k_1/k_2 = \text{Bioaccumulation Factor (BAF)} \tag{3}$$

Therefore, if one can determine the uptake and depuration rate constants, a BAF can be calculated even if a steady-state condition does not exist.

In this study, uptake (k_1) and depuration (k_2) rate constants were estimated using a computer program called BIOFAC developed by Blau and Agin (1978). BIOFAC is a nonlinear regression analysis program that generates rate constants from a set of sequential time-concentration data by fitting the data to Equation (2). The data are weighted by a normality preserving transformation to reflect any time-or concentration-related trends in variability. The rate constants were generated using all of the data from the uptake and depuration phases. The program treats the data in such a way that the effect of any lack of homogeneity in the data is eliminated. It provides not only the statistically best parameters, but also estimates their statistical variability. Input to the program included the number of sets of concentration-time data points, earthworm mercury tissue concentrations at each sample time period (ng g^{-1} dry weight earthworm), the duration of the exposure phase (28 days), and the concentration of mercury in the soil (μg kg^{-1} dry weight soil). Output included the

uptake rate constant (day^{-1}), depuration rate constant (day^{-1}), T(1/2) for clearance (day), bioaccumulation factor, and time to reach 90% of steady state (day). Although BIOFAC is an older computer program it has been used extensively for calculating bioaccumulation factors and bioconcentration factors, especially for experiments in which steady state was not achieved. For example, results from the BIOFAC program were recently used by the U.S. Department of Energy in its radiation dose modeling analysis for derivation of authorized limits for selected portions of the Hanford Reach National Monument (Napier *et al.*, 2004), by Health Canada for pesticide registration of methoxyfenozide (PMRA, 2004), and by the European Commission in its risk assessment report on benzene, C$_{10-13}$ alkyl derivatives (European Commission, 1999).

After application of the model to the various datasets, it was found that a depuration rate constant could not be determined in the low T-Hg or background T-Hg earthworms because no difference occurred in the mean T-Hg concentrations during the depuration phase in these treatments. Likewise, a depuration rate constant could not be determined in the background MMHg earthworms because uptake occurred throughout the 42-day exposure period since earthworms were transferred to background soils with the same mercury concentration during the depuration phase. The uptake and depuration rate constants were used to estimate the bioaccumulation factor (BAF) using Equation (3) for each data set where depuration rate constants could be determined.

In order to determine whether earthworm mercury tissue body burdens were significantly different between the treatment soils and the background soil, an analysis of variance (ANOVA) followed by a Dunnett's Test was conducted on the day 28 data for both T-Hg and MMHg. Tissue data were natural log transformed in order to satisfy normality and homogeneity of variance requirements. Alpha was set at 0.05 for all tests.

3. Results and Discussion

3.1. CHEMICAL CHARACTERISTICS OF THE SOILS

The general chemical characteristics of the four soils are summarized in Table I. The average concentration of T-Hg in the high, intermediate, low, and background mercury soils was 11,542, 2,825, 156, and 85 μg kg^{-1} dry weight soil, respectively. The average concentration of MMHg in the high, intermediate, low, and background mercury soils was 7.35, 2.56, 1.48, and 1.12 μg kg^{-1} dry weight soil, respectively. The average concentrations of T-Hg and MMHg in the study soils are the means of four replicates analyzed at day 0 and four replicates analyzed at day 28 of the study. No difference occurred in T-Hg and MMHG concentrations at day 0 and day 28; thus, the concentrations were averaged. The concentration of T-Hg and MMHg in

TABLE I

Characteristics of the soils used in the assays[a]

Analyte	High mercury soil	Intermediate mercury soil	Low mercury soil	Background mercury soil
T-Hg	11,542	2,825	156	85
MMHg	7.35	2.56	1.48	1.12
Ammonia (as N)	15.8	7.6	5.9	6.4
Cation exchange capacity	17.3	16.4	17.1	13.8
Clay (%)	14.6	13.2	12.2	6.7
Silt (%)	39.8	42.0	41.0	43.2
Sand (%)	45.6	44.8	46.8	50.1
Moisture (%)	47.4	47.2	47.6	47.0
Nitrate + Nitrite (as N)	13.3	10.8	10.2	13.1
pH	5.7–5.9	5.5–6.0	5.7–5.9	5.5–6.0
Total Kjeldahl nitrogen	396	206	233	597
Total organic carbon	31800	23300	26900	42400

[a]All units in mg kg^{-1} dry weight except for mercury concentrations (μg kg^{-1} dry weight), cation exchange capacity (meq/100 g), grain size (%), moisture (%), and pH (standard units).

the peat moss used to culture the earthworms was 35 and 0.42 μg kg^{-1} dry weight peat moss, respectively.

In addition to mercury, several other heavy metals (aluminum, antimony, copper, lead, manganese, selenium, and zinc) detected in one or more of the four soils exceeded U.S. National Oceanic and Atmospheric Administration's background levels for soils (Buckman, 1999). In all cases, the metals were <10% above background. With the exception of bis (2-ethylhexyl) phthalate in the high and intermediate soils, no other base neutral U.S. Environmental Protection Agency priority pollutants were found in the soils (U.S. Congress, 1972). No priority pollutant volatile organics, acid extractables, organophosphorous pesticides, chlorinated pesticides and herbicides, or nitroaromatic and nitramine explosives were found in the soils at their method detection limits.

3.2. SURVIVAL AND GROWTH

Percent survival of all replicates combined at the end of the 42-day exposure in the high, intermediate, low, and background mercury soils were 95.5, 99.5, 98.8, and 100%, respectively. Growth was linear in both the uptake and depuration phases in all exposures. An analysis of covariance showed that no difference in growth occurred between concentrations. The average growth rate of the earthworms was 5.1 and 6.1 mg week^{-1} dry weight in the uptake and depuration phases, respectively. The average growth rates of the earthworms in the current study were slightly

lower than the rates for *E. fetida* in the studies by Jeffries and Audsley (1988) and Neuhauser *et al.* (1980) that used growth media (pig and horse manure) with much higher organic carbon content. The high survival and linear growth in all soil treatments indicate that the mercury concentrations were not toxic to the earthworms over the exposure duration used in the study.

3.3. UPTAKE OF T-HG AND MMHG

The body burdens of both T-Hg and MMHg in *E. fetida* exposed to the three contaminated soils and the background mercury soil increased over the 28-d uptake period (Figures 1 and 2). The shape of the four uptake curves did not differ substantially among treatments for T-Hg, which indicates that the kinetics controlling uptake, were similar at all soil concentrations. Likewise, the shape of the curves did not differ among treatments for MMHg. The uptake rate constants (k_1), where they could be estimated, were essentially the same for T-Hg; they varied slightly for MMHg (Table II). A comparison of earthworm mercury body burdens at the
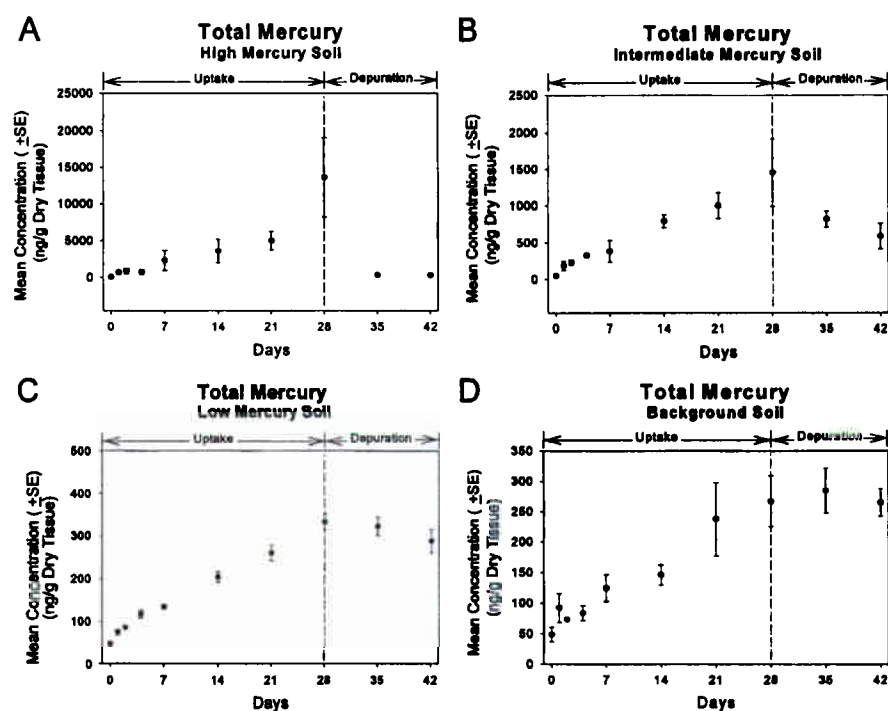

Figure 1. Total mercury uptake and depuration in the earthworm exposed to the high (A), intermediate (B), low (C), and background mercury (D) soils. Each data point is the mean ± SE of four replicates.
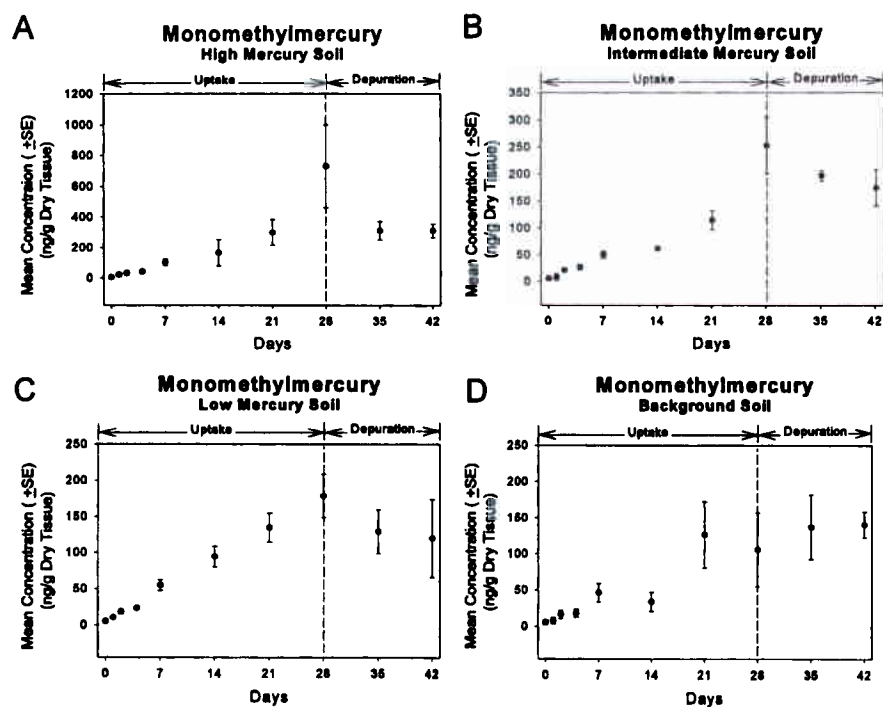
Figure 2. Monomethylmercury uptake and depuration in the earthworm exposed to the high (A), intermediate (B), low (C), and background mercury (D) soils. Each data point is the mean ± SE of four replicates.

high, intermediate, and low T-Hg treatments to the background mercury treatment showed that uptake was significantly greater in the high and intermediate T-Hg treatments relative to the background treatments. No difference occurred between the low contaminated and background mercury treatments for T-Hg. This is most likely due to the small difference in T-Hg concentrations between the low contaminated soil and the background mercury soil relative to the higher mercury concentrations in the other two soils. A comparison of the high, intermediate, and low MMHg treatments to the background mercury treatment showed that uptake was significantly greater in the high, intermediate, and low MMHg treatments relative to the background treatments.

A steady state did not appear to occur in earthworms during the 28-day uptake period for either T-Hg or MMHg in the high, intermediate, or low mercury soils. The time to 90% steady state for T-Hg in *E. fetida* was estimated to be 40 and 41 days, respectively, in the high and intermediate contaminated soils (Table II). A steady state did seem to occur in the T-Hg earthworms exposed to the background mercury

TABLE II

Summary of the uptake and depuration parameters for *Eisenia fetida* in the study soils. Standard deviations given in parentheses

parameter	High mercury soil	Intermediate mercury soil	Low mercury soil	Background mercury soil
Total mercury				
k_1 (day^{-1})	0.04 ($\pm$0.006)	0.03 ($\pm$0.003)	[a]	[a]
k_2 (day^{-1})	0.06 ($\pm$0.014)	0.06 ($\pm$0.008)	[a]	[a]
BAF	0.7 ($\pm$0.21)	0.6 ($\pm$0.96)	3.1[b]	2.1[b]
Time to 90% steady state (days)	40 ($\pm$9.5)	41 ($\pm$5.7)	28–35[c]	28–35[c]
Monomethylmercury				
k_1 (day^{-1})	2.33 ($\pm$0.270)	2.76 ($\pm$0.289)	5.57 ($\pm$0.360)	[d]
k_2 (day^{-1})	0.01 ($\pm$0.010)	0.02 ($\pm$0.006)	0.02 ($\pm$0.005)	[d]
BAF	175 ($\pm$130.3)	184 ($\pm$192.8)	234 ($\pm$47.9)	249[e]
Time to 90% steady state (days)	172 ($\pm$127.1)	192 ($\pm$164.7)	97 ($\pm$18.8)	[d]

[a]Value could not be determined because no depuration occurred.
[b]Estimate from average earthworm mercury concentration on day 28/soil mercury concentration.
[c]Time to steady state estimated from Figure 1.
[d]Value could not be determined because uptake was almost linear over the 42-day exposure period.
[e]MMHg concentration assumed to be equal to the T-Hg concentration at steady state.

soil after day 28 (Figure 1D). As discussed previously, the background treatment earthworms were exposed to a constant concentration of T-Hg for 42 days since they were transferred to background soil with the same mercury concentration for depuration. An apparent steady state appears to have been reached in the earthworms exposed to the low mercury soil; no difference occurred in T-Hg body burden between day 28 and 14 days of depuration (Figure 1C). This is probably a function of the similarity in mercury concentrations between the low and background soils. Although time to 90% steady state could not be calculated by the BIOFAC program since there appeared to be no or little depuration for these two soils, it appears from the plots (Figure 1C and D) that steady state was reached somewhere between day 28 and 35. The times to 90% steady state for MMHg were estimated to be 172, 192, and 97 days, respectively, in the high, intermediate, and low mercury soils (Table II). The estimates for 90% steady state were quite variable for MMHg (see standard deviations in Table II) as compared to the estimates for T-Hg. Monomethylmercury uptake in the background soil appeared almost linear .in *E. fetida* over the 42-d exposure and depuration experiment; thus, steady state could not be estimated (Table II).

The uptake of T-Hg over a 45-day exposure period has been studied by Helmke *et al.* (1979) in the earthworm *Aporrectodea tuberculata* using radioactive mercury ($^{203}Hg^{2+}$). The uptake curve was similar to the background mercury soil uptake curve in the current study. Uptake appeared to be near steady state by day 45, which is similar to the steady state values observed in this study (Table II). No comparable data are available in the literature for MMHg.

3.4. DEPURATION OF T-HG AND MMHG

Tissue concentrations of T-Hg in *E. fetida* at the high and intermediate soil concentrations decreased between 7 and 14 days of the depuration period when the animals were placed in the background mercury soil (Figures 1A and 1B). A significant decrease did not occur during depuration in the earthworms exposed to T-Hg in the low mercury soil; therefore, the BIOFAC program could not calculate a depuration rate constant (Figure 1C; Table II). Total mercury appeared to increase in *E. fetida* in the background mercury soil until it approached a steady state somewhere between day 28 and day 35; no elimination occurred (Figure 1D). As discussed earlier, this was most likely a function of these earthworms being transferred to fresh background soil during the depuration phase that had the same concentration as during the uptake phase. Thus in this background soil, this was, in effect, a 42-day uptake experiment. Monomethylmercury concentrations in the tissues of *E. fetida* at the high, intermediate, and low soil concentrations decreased between 7 and 14 days of depuration when the earthworms were placed in background mercury soil (Figure 2). No depuration occurred in the earthworms exposed to background MMHg; uptake continued during the 42-day exposure period (Figure 2D).

Neuhauser *et al.* (1995) have suggested that depuration rates for certain metals (i.e., copper, lead, and nickel) may increase as soil concentrations increase. The depuration rate constants (k_2) for T-Hg in this study were 0.06 at both the high (11,542 $\mu g \, kg^{-1}$ dry weight soil) and intermediate (2,825 $\mu g \, kg^{-1}$) soil concentrations (Table II). The depuration rate constants for MMHg were 0.01, 0.02, and 0.02 at the high (7.35 $\mu g \, kg^{-1}$ dry weight soil), intermediate (2.56 $\mu g \, kg^{-1}$), and low (1.48 $\mu g \, kg^{-1}$) soil concentrations, respectively. The data in the current study suggest that depuration rates do not increase at higher soil concentrations. The discrepancy between the two studies may be due to the fact that the depuration rates were determined in the current study over a 14-day period while the depuration kinetics were determined for periods up to 112-days in the Neuhauser *et al.* (1995) study where greater depuration occurred.

3.5. BIOACCUMULATION OF T-HG AND MMHG

The bioaccumulation factors for T-Hg in the high and intermediate mercury soils were estimated to be 0.7 and 0.6, respectively (Table II). The T-Hg BAFs for the low and background mercury earthworms were estimated to be 3.1 and 2.1, respectively.

These last two BAFs were estimated based on earthworm T-Hg tissue concentrations after 28 days when they appeared to be approaching steady state. The BAFs for MMHg in the high, intermediate, and low treatments were 175, 184, and 232, respectively. Since MMHg bioaccumulation appeared to increase linearly throughout the 42-day exposure period, the worst-case assumption was made that the MMHg concentration in the background earthworms eventually comprised 100% of the T-Hg concentration in the background earthworms at steady state. Using this assumption, the BCF for MMHg in the background soil would be 249.

The BAFs for T-Hg in this study were slightly larger for *E. fetida* exposed to the background (85 μg kg^{-1} dry weight soil) and low background mercury soils (156 μg kg^{-1}) (BAFs = 3.1 and 2.1) than the earthworms exposed to the intermediate (2,825 μg kg^{-1}) and high mercury soils (11,542 μg kg^{-1}) (BAFs = 0.6 and 0.7) (Table II). The absolute concentrations of T-Hg bioaccumulated by the earthworms, however, were larger at the higher soil concentrations. Larger BAFs in low T-Hg soils relative to lower BAFs in soils containing higher concentrations of T-Hg have been reported in other studies. For example, Sample *et al.* (1999) developed a regression model of T-Hg concentration in earthworms versus T-Hg concentration in the soil for earthworm data taken from several field studies. The regression showed that as the concentrations of T-Hg in the soil increased above $\sim$1 mg kg^{-1} dry weight soil, BAFs were <1. BAFs were >1 at soil concentrations below $\sim$1 mg kg^{-1}.

A number of explanations have been proposed to explain why most heavy metals do not continue to bioaccumulate in earthworms taken from the field when soil concentrations are high. Metal bioavailability in soils and the physiological regulation of metals by the organism appear to be most important. The bioavailability of several metals (e.g., copper, lead, nickel, and zinc) has been shown to decrease in natural soils as they age (Lock and Janssen, 2003; Lanno *et al.*, 2004). The soils used in the current study had aged approximately 30 yeas after mercury contamination was stopped. The most important soil characteristics that have been shown to influence metal bioavailability to earthworms are pH, organic matter content, cation exchange capacity, and calcium concentration. Of the above factors, pH appears to be the most important and has been shown to modulate pore water-mediated uptake of certain metals in earthworms (e.g., Ma *et al.*, 1983; Janssen *et al.*, 1997; Peijnenburg *et al.*, 1999a,b). pH was held constant (range 5.5–6.0) in the current study to minimize potential desorption/adsorption processes that may influence the bioavailability of T-Hg and MMHg. Sijm *et al.* (2000) have suggested that metals may be less available as the total organic content (TOC) of soil increases. Total organic carbon in this study did not appear to be correlated with the bioavailability of T-Hg or MMHg. The highest BAF for T-Hg (3.1) and MMHg (249) occurred in the background soil that had the highest TOC concentration (42,400 mg kg^{-1} dry weight soil) (Table I). The lowest BAF for T-Hg (0.6) occurred in earthworms in the intermediate soil that had the lowest TOC concentration (23,000 mg kg^{-1} dry weight). The BAF for MMHg was 184 in the intermediate soil.

Physiological regulation by the organism has been shown to be a mechanism that can regulate internal concentrations of essential metals, such as copper, nickel, and zinc when the concentrations in the soil are high (e.g., Ireland, 1979; Fleckenstein and Graff, 1982; Svendsen and Weeks, 1997; Peijnenburg et al., 1999a). Total mercury and MMHg tissue concentrations were greatest in the earthworms exposed to soils at the higher mercury concentrations. Thus, physiological regulation of T-Hg and MMHg did not appear to occur in the present study. Lock and Janssen (2001a,b) have suggested that earthworms can detoxify nonessential metals such as cadmium, although the process is very slow. Total mercury concentrations were estimated to reach 90% steady state at 40–41 days in the high and intermediate soil exposures; steady state appeared to occur between 28 and 35 days in the lower two concentrations. The estimated times to 90% steady state were much longer for MMHg (97–192 days). If mercury detoxification occurred as suggested for cadmium by Lock and Janssen (2001a,b), detoxification of inorganic mercury (T-Hg) occurred much more rapidly than MMHg.

As was case for T-Hg, the BAFs for MMHg were also larger in earthworms exposed to the lower mercury soils than in the organisms in the higher contaminated soils (Table II). The MMHg BAFs were 249, 234, 184, and 175 in soils containing 1.12, 1.48, 2.56, and 7.35 μg kg^{-1} dry weight soil. The absolute concentration of MMHg bioaccumulated in E. fetida was highest in the earthworms exposed to the high mercury soil and lowest in the background mercury soil. Beyer et al. (1985) also demonstrated that MMHg can bioaccumulate in earthworms. The BAFs for MMHg in the Beyer et al. (1985) earthworms (E. fetida) exposed up to 84 days range from 84–91 when the data are corrected to dry weight earthworm and dry weight soil. The BAFs were lower than those predicted from the current study. Based on the estimated time to 90% steady state in the current study (Table II), the earthworms in the Beyer et al. (1985) study did not appear to be at steady state. Thus, the BAFs would be expected to be lower than those predicted at steady state in the current study.

The BAFs for MMHg were 75- to 300-fold larger than those for T-Hg in the study soils. One may speculate that the bioaccumulation of MMHg is different from T-Hg because it is an organic compound that may partition to lipid. Based on the octanol-water partition coefficient for MMHg (log K_{ow} ranges from ~1.6 at pH 4 down to ~0.4 at pH 8) (Major and Rosenblatt, 1991), some bioaccumulation should occur in the lipid compartment. The role of the lipid compartment for MMHg is minimal, however, compared to hydrophobic organic chemicals, e.g., chlorinated pesticides, with large K_{ow}s (>6) (Jager, 1998). If the role of the lipid compartment is minimal in E. fetida, one may argue that other mechanisms are involved in MMHg accumulation. One possibility that might be further explored is the potential for methylation of divalent mercury to MMHg via bacteria in the digestive tract of the earthworm. The concentrations of MMHg in each of the four soil treatments did not change over the course of the 28-day uptake period. Thus, the microorganisms

in the soil were not responsible for the MMHg increases observed in the tissues of the earthworms during the uptake phase of the study.

The BAFs determined in this study for T-Hg are consistent with those in the published literature which range from <1 up to ~10. Prior to this study, no BAFs were available for MMHg in earthworms. The estimated MMHg BAFs for *E. fetida* in this study ranged from 175 to 249. Before these values are considered for definitive use in environmental risk assessments, a number of variables should be considered. The current BAFs have a high degree of uncertainty associated with the estimates based on the standard deviation of the values (Table II). Longer exposures may reduce the variation and better define the BAFs. Ma (2004) has made the point that *E. fetida* is adapted to living in compost heaps and is not normally found in field soils. Although *E. fetida* was exposed to natural field soil contaminated with mercury, it is likely that MMHg BAFs from other groups of oligochaetes (i.e., epigenic, endogenic, and anecic earthworms) that normally reside in field soils may differ from those estimated for *E. fetida* in this evaluation. Finally, the bioavailability of organic mercury in different soil types should be considered before the MMHg BAFs are used routinely to estimate MMHg bioaccumulation in earthworms and subsequent bioaccumulation in piscivorous wildlife in mercury-contaminated soil risk assessments.

Acknowledgements

References

ASTM (American Society for Testing and Materials): 1998, 'Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia fetida*', ASTM Designation E 1676-97, in *1998 Annual Book of ASTM Standards*, Vol. 11.05, American Society for Testing and Materials, Philadelphia, PA, USA, pp. 1056–1074.

Beyer, W. N., Cromartie, E. and Moment, G. B.: 1985, 'Accumulation of methylmercury in the earthworm, *Eisenia foetida*, and its Effect on Regeneration', *Bull. Environ. Contam. Toxicol.* 35, 157–162.

Blau, G. E. and Agin, G. L.: 1978, *A Users Manual for BIOFAC: A Computer Program for Characterizing the Rates of Uptake and Clearance of Chemicals in Aquatic Organisms*, Dow Chemical Co., Midland, MI, USA.

Boudou, A. and Ribeyre, F.: 1997, 'Mercury in the food web: Accumulation and transfer mechanisms', in A. Sigel and H. Sigel (eds), *Metal Ions in Biological Systems, Vol. 34, Mercury and its Effects on Environment and Biology*, Marcel Dekker, Inc., NY, USA, pp. 289–319.

Buckman, M. F.: 1999, '*NOAA Screening Quick Reference Tables*', HAZMAT Rep. 99-1', Coastal Protection and Restoration Division, National Oceanic and Atmospheric Administration, Seattle, WA, USA, 11 pp.

Bull, K. R., Roberts, R. D., Inskip, M. J. and Goodman, G. T.: 1977, 'Mercury concentrations in soil, grass, earthworms and small mammals near an industrial emission source', *Environ. Pollut.* **12**, 135–140.

Cocking, D., Hayes, R., King, M. L., Rohrer, M. J., Thomas, R. and Ward, D.: 1991, 'Compartmentalization of mercury in biotic components of terrestrial flood plain ecosystems adjacent to the South River at Waynesboro, Va.', *Water Air Soil Pollut.* **57–58**, 159–170.

Cocking, D., King, M. L., Ritchie, L. and Hayes, R.: 1994, 'Earthworm Bioaccumulation of Mercury from Contaminated Flood Plain Soils', in C. J. Watras and J. W. Huckabee (eds), *Mercury Pollution Integration and Synthesis*, Lewis Publishers, Boca Raton, FL, USA, pp. 381–395.

European Commission: 1999, 'European Union Risk Assessment Report benzene, C_{10-13} alkyl derives, Volume 3', EUR 19011, in B. G. Hansen, S. J. Munn, G. Schoening, M. Luotamo, A. van Haelst, C. J. A. Heidorn, Pellegrini, R. Allanou and H. Loonen (eds), Office for Official Publications of the European Communities, Luxembourg.

Fischer, E. and Koszorus, L.: 1992, 'Sublethal effects, accumulation capacities and elimination rates of As, Hg and Se in the Manure Worm, *Eisenia fetida* (Oligochaeta, Lumbricidae)', *Pedobiologia* **36**, 172–178.

Fleckenstein, J. and Graff, O.: 1982, 'Schwermetallaufnahme aus Mullkompost Durch den Regenwurm *Eisenia foetida* (Savigny 1826)', Landbauforschung Volkenrode **32**, 198–202.

Gibbs, M. H., Wicker, L. F. and Stewart, A. J.: 1996, 'A method for assessing sublethal effects of contaminants in soils to the earthworm, *Eisenia fetida*', *Environ. Toxicol. Chem.* **15**, 360–368.

Helmke, P. A., Robarge, W. P., Korotev, R. L. and Schomberg, P. J.: 1979, 'Effects of soil-applied sludge on concentrations of elements in earthworms', *J. Environ. Qual.* **8**, 322–327.

Ireland, M. P.: 1979, 'Metal accumulation by the earthworms *Lumbricus rubellus*, *Dendrobaena veneta* and *Eiseniella tetraedra* living in heavy metal polluted sites', *Environ. Pollut.* **19**, 201–206.

Jager, T.: 1998, 'Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta)', *Environ. Toxicol. Chem.* **17**, 2080–2090.

Janssen, R. P. T., Posthuma, L., Baerselman, R., Den Hollander, H. A., Van Veen, R. P. M. and Peijnenburg, W. J. G. M.: 1997, 'Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms', *Environ. Toxicol. Chem.* **12**, 2479–2488.

Jeffries, J. R. and Audsley, E.: 1988, 'A Population Model for the Earthworm *Eisenia foetida*', in C. A. Edwards and E. F. Neuhauser (eds), *Earthworms in Waste and Environmental Management*, SPB Acad Publ bv The Hague, The Netherlands, pp. 119–134.

Keating, M. H., Mahaffey, K. R., Shoeny, R., Rice, G. E., Bulluck, O. R., Ambrose, R. B., Jr., Swartout, J. and Nichols, J. W.: 1997, '*Mercury Study Report to Congress, Vol. 1: Executive Summary*', EPA-452/H-97-003, U.S. Environmental Protection Agency, Washington, DC, USA.

Lanno, R., Wells, J., Conder, J., Bradham, K. and Basta, N.: 2004, 'The bioavailability of chemicals in soil for earthworms', *Ecotoxicol. Environ. Safety* **57**, 39–47.

Lock, K. and Janssen, C. R.: 2001a, 'Zinc and cadmium body burdens in terrestrial oligochates: use and significance in environmental risk assessment', *Environ. Toxicol. Chem.* **20**, 2067–2072.

Lock, K. and Janssen, C. R.: 2001b, 'Cadmium toxicity for terrestrial invertebrates: Taking soil parameters affecting bioavailability into account', *Ecotoxicology* **10**, 315–322.

Lock, K. and Janssen, C. R.: 2003, 'Influence of aging on metal availability in soils', *Rev. Environ. Contam. Toxicol.* **178**, 1–21.

Loux, N. T.: 1998, 'An Assessment of mercury-species-dependent binding with natural organic carbon', *Chem. Speciation Bioavailability* **10**, 127–136.

Ma, W.-C.: 2004, 'Estimating heavy metal accumulation in oligochaete earthworms: A meta-analysis of field data', *Environ. Contam. Toxicol.* **72**, 663–670.

Ma, W.-C., Edelman, T., Van Beersum, I. and Jans, T.: 1983, 'Uptake of cadmium, zinc, lead, and copper by earthworms near a zinc-smelting complex. Influence of soil pH and organic matter', *Bull. Environ. Contam. Toxicol.* **30**, 424–427.

Major, M. A. and Rosenblatt, D. H.: 1991, 'The octanol/water partition coefficient of methylmercuric chloride and methylmercuric hydroxide in pure water and salt solutions', *Environ. Toxicol. Chem.* **10**, 5–8.

Munthre, J.: 1994, 'The Atmospheric Chemistry of Mercury: Kinetic Studies of Redox Reactions', in Watras, C. J. and Huckabee, J. W. (eds), *Mercury Pollution Integration and Synthesis,* Lewis Publishers, Boca Raton, FL, USA, pp. 273–279.

Napier, B. A., Kennedy, W. E., Ikenberry, T. A., Hunacek, M. M. and Kennedy, A. M.: 2004, 'Technical Basis for the Derivation of Authorized Limits for Units of the Hanford Reach National Monument', PNNL-14531, Pacific Northwest National Laboratory, Richland, WA, USA.

Neuhauser, E. F., Cukic, Z. V., Malecki, M. R., Loehr, R. C. and Durkin, P. A.: 1995, 'Bioconcentration and biokinetics of heavy metals in the earthworm', *Environ. Pollut.* **89**, 293–301.

Neuhauser, E. F., Hartenstein, R. and Kaplan, D. L.: 1980, 'Growth of the earthworm *Eisenia foetida* in relation to population density and food rationing', *Oikos* **35**, 93–98.

Newman, M. C. and Unger, M. A.: 2003, *Fundamentals of Ecotoxicology (2nd ed.),* Lewis Publishers, Boca Raton, FL, USA.

Peijnenburg, W. J. G. M., Baerselman, R., de Groot, A. C., Jager, T., Posthuma, L. and Van Veen, R. P. M.: 1999a, 'Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the oligochaete *Eisenia andrei*', *Ecotoxicol. Environ. Safety* **44**, 294–310.

Peijnenburg, W. J. G. M., Posthuma, L., Zweers, P. G. P. C., Baerselman, R., de Groot, A. C., Van Veen, R. P. M. and Jager, T.: 1999b, 'Prediction of metal bioavailability in Dutch field soils for the oligochaete *Enchytraeus crypticus*', *Ecotoxicol. Environ. Safety* **43**, 170–186.

PMRA: 2004, 'Regulatory Note, Methoxyfenozide', REG2004-08, Pest Management Regulatory Agency, Health Canada, Ottawa, Ontario, Canada.

Sample, B. E., Suter, G. W., II, Beauchamp, J. J. and Efroymson, R. A.: 1999, 'Literature-derived bioaccumulation models for earthworms: Development and validation', *Environ. Toxicol. Chem.* **18**, 2110–2120.

Seigneur, C., Lohman, K., Pai, P., Heim, K., Mitchell, D. and Levin, L.: 1999, 'Uncertainty analysis of regional mercury exposure', *Water Air Soil Pollut.* **112**, 151–162.

Sijm, D., Kraaij, R. and Belfroid, A.: 2000, 'Bioavailability in soil or sediment: Exposure of different organisms and approaches to study it', *Environ. Pollut.* **108**, 113–119.

Stein, E. D., Cohen, Y. and Winer, A. M.: 1996, 'Environmental distribution and transformation of mercury compounds', *Crit. Rev. Environ. Sci. Technol.* **26**, 1–43.

Svendsen, C. and Weeks, J. M.: 1997, 'Relevance and applicability of a simple earthworm biomarker of copper exposure. II. Validation and applicability under field conditions in a mesocosm experiment with *Lumbricus rubellus*', *Ecotoxicol. Environ. Safety* **36**, 80–88.

Talmage, S. S. and Walton, B. T.: 1993, 'Food chain transfer and potential renal toxicity of mercury to small mammals at a contaminated terrestrial field site', *Ecotoxicology* **2**, 243–256.

U.S. Congress: 1972, *'Federal Water Pollution Control Act'*, 33 U.S.C. pp. 1251–1376, Pub. L. No 95-500, Sect. 307, 86 Stat. 816, U.S. Congress, Washington, DC, USA.

van Gestel, C. A. M., Dirven-van Breemen, E. M. and Baerselman, R.: 1992, 'Influence of environmental conditions on the growth and reproduction of the earthworm *Eisenia andrei* in an artificial soil substrate', *Hydrobiologia* **36**, 109–120.

Wolfe, M. F., Schwarzbach, S. and Sulaiman, R. A.: 1998, 'Effect of mercury on wildlife: A comprehensive review', *Environ. Toxicol. Chem.* **17**, 146–160.